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Prevalence and Inhibition of Microbial Load on Chicken Eggs with Special References to Egg Quality and Hatchability

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Abstract: A total of 304 random chicken egg samples obtained from layers of Balady and Battery systems. 144 of chicken Balady and Battery eggs (72 of each sample were divided into 18 batches) were used for bacteriological evaluation. Enterobactriacae (94.4, 33.3 and 27.7%), Salmonella (77, 29 and 22%), *E.coli* (44, 0 and 22%*)* and *Staphylococcus aureus (*100, 97 and 98%) were detected in shell, albumen and yolk in Balady eggs, respectively. Battery system showed lower incidence in Enterobactriacae (50, 16.6 and 22%), Salmonella (41.6, 8 and 0%), *E.coli* (27.7, 9 and 19%) and *Staphylococcus aureus* (100, 83 and 95%), respectively. Results concluded that the system in which the hens are housed contribute in rate of contamination of eggs. *Staphylococcus aureus* showed a higher prevalence rate compared to other pathogens in both laying systems. About 160 Balady eggs were selected for studying the effect of different pathogen inhibitors, 80 eggs for detecting preservation after 21 days at room temperature and the other 80 fertile Balady eggs for detecting hatchability and mortality. The efficacy of application of different pathogen inhibitors as Propionic acid at different concentration 10, 30, 50, 70 and 100%, Hydrogen Peroxides (H_2O_2) 3% and Virkon S 1% on eggs were recorded. Propionic acid10%, Virkon S and H_2O_2 showed nearly similar significant inhibitory effect on pathogens on egg shells ranged from 86 to 100%, albumin from 33.4 to 100% and yolk from 34.3 to100%, while 30% Propionic acid has highly significant inhibitory effect on pathogen load ranged from 99.8 to100%. About 30% propionic acid concentration had a preservative effect on table eggs for 21 days at room temperature and increasing hatching percent up to 90% and lowering embryonic mortality to10% in fertile eggs. The findings of this study indicate that 30% Propionic acid may be considered as a favorable disinfectant agent for the egg shell spraying.

Keywords: Egg Pathogen, Bacterial Inhibitors, Egg Quality and Hatchability

Introduction

Eggs can fully meet the requirements of all nutrients necessary for human development and life functions. At the same time, many nutrient substances present in egg create an excellent environment for the development of different microflora, including pathogenic microorganisms (Bufano-Nancy, 2000; Griffiths, 2005). Shell eggs without cracks have many natural barriers that prevent bacteria from entering and growing (Edema and Atayese, 2006). The ways of microbial contamination are vertical and horizontal transmissions Cox *et al*. (2000; Ellen *et al*., 2000; Smith *et al*., 2000). Pathogen penetration through the egg shell affect several aspects in keeping quality, hatching like early embryonic mortality, egg yolk infection and mortality before hatching (Berrang *et al*., 1999).

Salmonella can be regarded as two types of infections. The first is primarily of importance for public health by causing food borne illness. The other type causes severe disease leads to great economic losses in poultry industry (Anbessa and Shiferaw, 2013; CDC, 2014; SVA, 2014).

Egg shell contamination is the main reason for *E. coli* infection. Poor hatcher sanitation can leave a residue of

E. coli from the previous hatch leads to yolk infections which occur during hatching process (Eric, 2011).

Staphylococcus aureus dominated on the shell and in yolk compared to egg white. The degree of contamination with these bacteria regarding to the source of eggs (Stępień-Pyśniak *et al*., 2009; Shareef *et al*., 2009).

Egg treatment with disinfectant is often used by the poultry industry to improve day-old chick quality, hatchability and keeping quality by reducing the microbial population on the egg shell surface Enev *et al*. (2005; Olayemi and Adetunji, 2013). Also, pathogen could penetrate egg during storage in storerooms, thus deteriorating their quality (Pavlov *et al*., 2006; Ivanov, 2008). For further safety, government regulations in some countries require special good hygienic practice and application of suitable detergent and sanitizer (Deeks, 2005; Baychev and Karadjov, 2006; Edema and Atayese, 2006; Best, 2007; Luc, 2007; Madec, 2007).

Therefore, the main objective of the present study was to:

- Detection and identification of some pathogenic bacteria present in eggs comparing different rearing systems
- Qualification and quantification the effect of different disinfectants on both edible and hatching eggs for studying their role in improving keeping quality and hatchability

Material and Methods

Collection of Samples

A total of 304 random egg samples, 144 of chicken Balady and Battery eggs (72 of each sample were divided into 18 batches each contain 4 eggs represented as one sample) were used for bacteriological evaluation. Every 4 eggs were placed in a plastic bag and transferred to the laboratory without delay for microbiological examination.

About 160 Balady eggs were selected for studying the effect of different pathogen inhibitors, 80 eggs for detecting preservation after 21 days at room temperature and the other 80 fertile Balady eggs for detecting hatchability and mortality.

Preparation of Samples

Egg samples were prepared for bacteriological examination according to (AOAC, 2000).

Bacteriological Indices

Were carried before and after application of pathogenic inhibitors (Table 1) in some selected pathogens as follows:

- Total *Enterobactriacae* counts (APHA, 1992)
- Isolation of *E. coli* (APHA, 1992)
- Isolation of *Salmonella spp.* (USDA/FSIS, 1998)
- Isolation of *Staphylococcus* aureus (Finegold and Martin, 1982)

Determination of pH**:** Using digital pH meter Model: cd 713 Qingdao TLead International Company Ltd.Shandong.China.

Sensory Evaluation of Treated Egg According to Lawless and Hildegrade (2010)

Odor, taste and texture of raw and cooked eggs before and after application of pathogenic inhibitors were tested.

The Efficacy of Application of Pathogenic Inhibitors upon the Hatchability Embryonic Mortality of Fertile Eggs

About 80 fertile eggs were treated with H2O2, Virkon S and Propionic acid (10, 30, 50, 70 and 100%), non-treated served as controls. Fertile eggs confirmed on 7th day after incubation) only 80 fertile eggs (10/each) treatment were kept till hatching. Eggs were incubated in an automatic incubator with temperature 37.5±1°C and relative humidity 45-50% humidity for day 1-18, then 65% for the last few days. Eggs were automatically turned in interval of two hours.

Hatchability: Was expressed as percentages of total fertile eggs.

Effect of Disinfectants on Preservation of Table Eggs

About 70 table eggs (10/each) were treated with H2O2, Virkon S and Propionic acid (10, 30, 50, 70 and 100%) eggs, 10 non-treated eggs served as controls. All eggs were examined after 21 days of incubation at room temperature for measuring rate of preservation (Menezes *et al*., 2012; SPSS 20, 1989-2014).

Results

Results were clarified in Fig. 1-3 and Table 2.

Higher incidence of pathogen detected in Balady over Battery system. Shell was the superior in contamination.

Preservation and both hatchability rate and embryonic mortality of treated fertile eggs and were improved after using Propionic acid 30% followed by Propionic acid 10%, H_2O_2 and Virkon-S.

Table 1. Concentration of pathogenic inhibitors

Product class	PH	Dilutions	
Hydrogen Peroxide (H_2O_2)	7.5	3%	
Virkon S	6.2	1%	
Propionic acid	2.9	10%	(Prop.10%)
Propionic acid	2.8	30%	(Prop.30%)
Propionic acid	2.6	50%	(Prop.50%)
Propionic acid	2.4	70%	(Prop.70%)
Propionic acid	2.0	100%	(Prop. Conc).

Table 2. Quantitative and inhibitory percent of pathogenic inhibitors on bacterial load

	Shell			
Preparation	*Entero.bact.*	*Salm.*	*E.coli*	*Staph*
Control	$43\times10^{2a}\pm57.73$	1×10^{2a}	$14\times10^{2a}\pm56.73$	$5\times10^{5a}\pm115.47$
Inhibition%	0	0	0	0
H_2O_2	$2.3\times10^{2b}\pm0.57$	0^{b}	$10^{c}\pm0.6$	$7\times10^{4b}\pm11.54$
% of inhibition	94.7	100	99.3	86
Virkon S	$2.2\times10^{2b}\pm0.57$	0^{b}	0^{c}	$3\times10^{4c}\pm57.73$
Inhibition%	94.9	100	100	94
Prop.10	$2.5\times10^{2b}\pm0.57$	0^{b}	$1.6\times10^{2b}\pm5.6$	$6\times10^{3d}\pm1.15$
Inhibition%	94.2	100	89	99.8d
Prop.30%	0^{c}	0^{b}	0^{c}	$8\times10^{2d}\pm1.15$
Albumen				
Control	$2\times10^{2a}\pm5.7$	0	$1.2\times10^{2a}\pm11.5$	$4\times10^{2a}\pm2.88$
Inhibition%	0	0	0	0
H_2O_2	0^{b}	0	$0.8\times10^{2b}\pm2.88$	$1.2^{c}\times10\pm1.15$
% of inhibition	100	0	33.4	97
Virkon S	0^{b}	0	0^{c}	$3.1\times10^{b}\pm0.57$
Inhibition%	100	0	100	92.3
Prop.10	0^{b}	0	0^{c}	$1\times10^{c}\pm2.30$
Inhibition%	100	0	100	97.5
Prop.30%	0^{b}	0	0^{c}	0^{d}
Yolk				
Control	$2\times10^{2a}\pm17.32$	$6.4\times10^{2a}\pm28.86$	$3.8\times10^{a}\pm2.3$	$4\times10^{2a}\pm8.66$
Inhibition%	0	0	0	0
H_2O_2	$3\times10^{c}\pm2.3$	0^{b}	$2.5\times10^{b}\pm2.88$	$5\times10^{b}\pm2.3$
% of inhibition	85	100	34.3	87.5
Virkon S	$9\times10^{b}\pm11.54$	0^{b}	0^{c}	$1.3\times10^{d}\pm1.73$
Inhibition%	100	100	100	96.8
Prop.10	$1\times10^{cd}\pm2.3$	0^{b}	0^{c}	$3\times10^{c}\pm2.3$
Inhibition%	95	100	100	92.5
Prop.30%	0^{d}	0^{b}	0^{c}	0^{d}

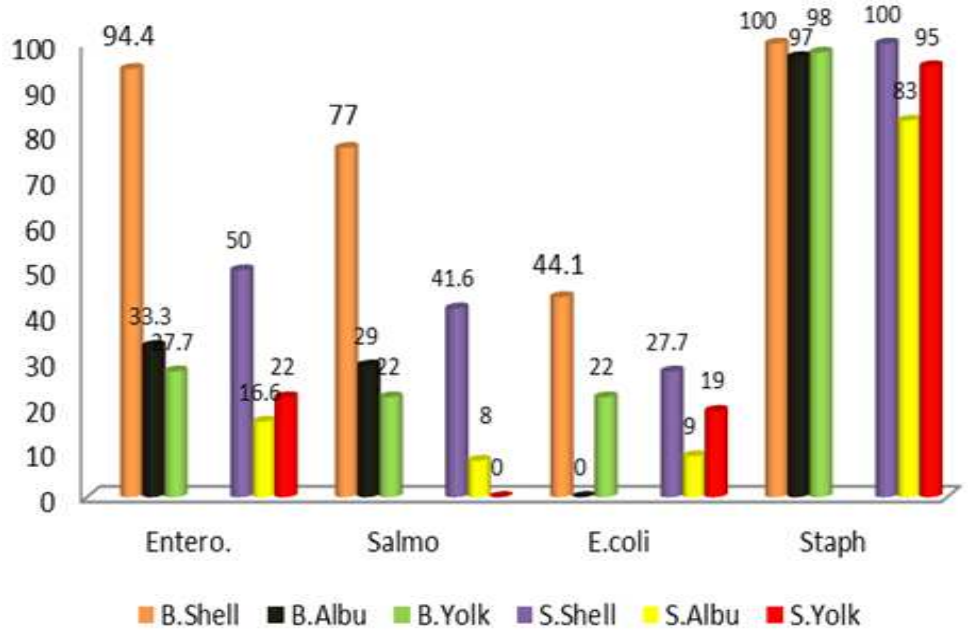

Fig. 1. Frequency distribution of pathogen in examined rearing systems *Balady (Shell-albumen-yolk) B.Shell-B.Albu-B. Yolk **Battary system((Shell- albumen-yolk) S.Shell-S.Albu- S.Yolk

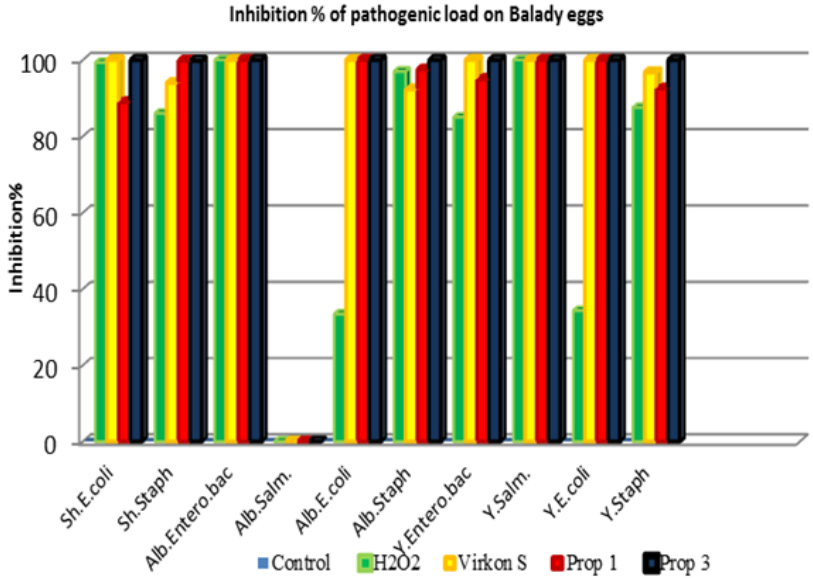

Fig. 2. Inhibitory percent of pathogenic inhibitors on bacterial load in Balady eggs

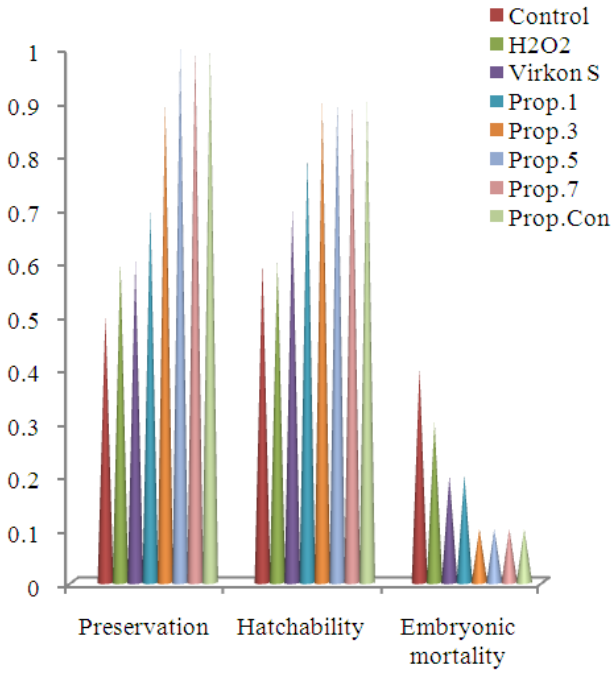

Fig. 3. Effect of different pathogenic inhibitors on table egg preservation, fertile egg hatching and embryonic mortality

Moreover, Propionic acid 50, 70 and 100% showed similar results as 30% propionic acid.

Discussion

Egg and egg products are the primary vehicles for the transmission of pathogenic microorganism to man and it is estimated that about 50% of the foodborne cases from the consumption of contaminated egg or egg products (Suba *et al*., 2005; Sayed *et al*., 2009). Eggs have physical and biological defense mechanisms to protect the embryo against invasion and multiplication of micro-organisms Jerzy and Dagmara (2009).

Influence of housing system on egg quality. The safety of eggs depends on the number of bacterial cells on shell and content of eggs for presence of factors that initiate pathogen multiplication (Ricke *et al*., 2001). The risk of illness resulting from consumption of contaminated eggs depends not only on the number of bacterial cells in eggs, but also on the type of bacteria (Bradshaw *et al*., 1990). Our data revealed a higher incidence of pathogen on shell of Balady over Battery system which may be attributed to poor sanitary conditions, initiating pathogen penetration through horizontal route and this result coincided with Smith *et al*. (2000); Knape *et al*. (2002); Theron *et al*. (2003). Our data indicated a highest incidence of pathogen on shell followed by albumen and finally yolk in both Balady and Battery system, these were attributed to the eggs temperature switch from 40°C to an ambient one during oviposition, creating a pressure gap through the egg shell to balance inside and outside pressure, the air will be "sucked" through the pores initiating entrance of most pathogen this was clarified by Turblin (2012). Moreover, our results proved lower incidence of pathogen in albumen than shell that might be due to the natural defense system of the egg in albumen which contains a number of proteins with demonstrated antimicrobial activities, such as lysozyme, bacterial cell lysis, metal binding and vitamin binding, prevent bacteria from entering and growing Edema and Atayese (2006). On contrary, Labaque *et al*. (2003); Jones *et al*.

(2004) and Abdul Aziz *et al*. (2012) indicated that higher prevalence and counts of bacteria on egg shell was relative to the egg contents.

Frequency distribution of pathogen in laying egg systems. Results given in Fig. 1, shows that the frequency *Enterobactriacae* in Balady eggs were 94.4, 33.3 and 27.7% while in Battery eggs the percentages were 50, 16.6 and 22% in shell, albumen and yolk respectively. *Enterobactriacae* were recorded to play a role in spoilage and food poisoning Stępień-Pyśniak (2010). Our results showed high incidence of *Enterobactriacae* in Balady eggs in compared to Battery eggs these attributed to the poor sanitary conditions, similar results were recorded by Carter and Cole Jr. (1990).

The incidence of *Salmonella spp. as* shows in Fig. 1 in both Balady and Battery eggs were 77, 29 and 22%; 41.6, 8 and 0% in shell, albumen and yolk respectively. Salmonella is of importance for public health by causing food borne illness in human beings and severe disease with economic importance in poultry industry (CDC, 2014; SVA, 2014). Lower incidence was recorded on the egg shells about (3.2%) by Jones *et al*. (2004), Rodenburg *et al*. (2006) and De Reu *et al*. (2007). Other researcher detected lowest incidence of Salmonella in table eggs with 0.07% in egg content and 0.4% in shell by Poppe *et al*. (1998), De Reu *et al*. (2006) who found 0.18% and Begum *et al*. (2010) who reported variable and very low incidence of Salmonella. On contrary, Favier *et al*. (2000); Anon (2004) and Abdul Aziz *et al*. (2012) failed to isolate *Salmonella spp*. these results variation could be attributed to different control measures applied against these bacteria.

In the present study, the data obtained showed that *E. coli* was isolated from shell and yolk of Balady eggs but not from albumen (44, 0 and 22%), while lower incidence detected in Battery system which were 27.5, 9 and 19% of shell, albumin and yolk, respectively. Nearly similar results were obtained by Adesiyun *et al*. (2005) who found this bacterium on 58.7% of shell and in 4.3% of egg contents of farm eggs also, they added that the frequency of *E. coli* founded in eggs depending on their rearing sources.

The incidence of *Staphylococcus aureus* as shown in Fig. 1 was 100, 97 and 98% in Balady egg samples while in Battery system was 100, 83 and 95% in shell, albumin and yolk, respectively. *Staphylococcus* aureus has been reported by Hazariwala *et al*. (2002) as an important cause of diseases in poultry. McCullagh *et al*. (1998) found *Staphylococcus aureus* as a common isolate from the clinically diseased broilers and as a cause of yolk sac infection in broilers also they found both *E. coli* and *Staphylococcus aureus* may cause chick mortality after hatching. Moreover, Stępień-Pyśniak *et al*. (2009) demonstrated that *Staphylococcus spp.* dominated in the yolks 38.8%, on the shells 58.9 and 2.5% in white of table eggs.

Variance in prevalence of bacterial contamination from shell and content might attributed to penetration rate. These results supported by Al-Ali *et al*. (2012) who found that Salmonella *spp.* were the highest in penetration rate followed by *Stapylococcus aureus* and *Escherichia coli* through egg pours.

Pathogenic inhibitors on eggs disinfectant preparations and concentrations need to be carefully scrutinized (Miguel *et al*., 2001). Hydrogen peroxide 3% concentrations are commonly used in the poultry industry. Figure 2 Illustrated that H_2O_2 showed significant reduction on quantitative pathogenic load on shell ranged from 86 to 100%, albumen from 33.4-to 100% and yolk from 34.3 to 100%. It was clarified that H_2O_2 was more effective in reduction on shell than albumen and yolk respectively. This may be attributed to the inability of H_2O_2 to invade egg through pores. Nearly similar finding were recorded by Sander and Wilson (1999) who demonstrated that 3% H_2O_2 was effective in reducing bacteria and Wells *et al*. (2011) who detected 3 log reductions in total bacterial count. Miguel *et al*. (2001) and Rodgers *et al*. (2001) recommended the use of H_2O_2 in poultry industry. Cox *et al*. (2000) found that Hydrogen peroxide is an effective chemical for the disinfection of fertile hatching eggs and does not adversely affect hatchability and also improve the hatching potential. Sheldon and Brake (1991), Padron (1995) Luc (2002) and Higgins *et al*. (2005) added that H_2O_2 showing no detrimental effects on both preservation and hatchability.

Virkon-S (1%) has a wide spectrum bactericidal, veridical, fungicidal and good safety characteristic AI (2004). Figure 2 showed that Virkon-S had a significant reduction on pathogenic load on shell ranged from 94 to 100%, albumen from 92.3 to 100% and yolk from 96.8 to 100%. However, Gasparini *et al*. (1995) found that Virkon-S is effective but the prolonged use may leads to resistant pathogens. This finding coincides with those of Sidhu *et al*. (2002; Moustafa Gehan *et al*., 2004; Ellen, 2006).

Propionic acid is a bactericide and fungicide, safely used in poultry farms Haque *et al*. (2009). Figure 2 showed that Propionic acid 10% had a significant reduction on pathogenic load on shell ranged from 89 to 100%, albumen from 97.5 to 100% and yolk from 92.5 to 100%.

Our results proved that 30% propionic acid showed the highest reduction percent ranged from 99.8 to 100%

on shell and content indicating high penetration availability. Similar inhibitory effect were recorded by using 50,70 and 100% concentration on shell, albumen and yolk, therefore 30% Propionic acid concentration was the lowest concentration having the highest significant inhibitory effect.

Figure 3 illustrated that spraying 30, 50, 70,100% of propionic acid showed the highest preservation effect which attributed to its inhibitory effect on bacterial load of table eggs. Propionic acid 30% was the lowest concentration having the highest preservation effect than H_2O_2 and Virkon-S. Similar data were recorded in both hatchability rate and embryonic mortality of treated fertile eggs; nearly similar data were detected by Higgins *et al*. (2005). Sensory evaluation of raw and cooked eggs not affected by spraying different pathogenic inhibitors. Our data indicated that pH (2.8-2.6-2.4- and 2) 30, 50, 70 and 100% of propionic acid, respectively, exhibits higher bactericidal activity this may be attributed to the Short chain of propionic acid which have been found to be efficacious in lowering the pH and thus don´t allow the pathogenic microorganisms to grow, similar results were detected by Luckstadt, (2005). Superiority of propionic acid over other preparations in its bactericidal activity could be explained in the light of time of dissociation, whereas propionic acid dissociate slowly, so the antimicrobial effect depends upon the dissociation constant (pKa) or pH., at which 50% of the total acid is undissociated. The undissociated part of the molecule is related to the antimicrobial effect through penetration into the microbial cells (Davidson and Taylor, 2007).

Conclusion

From the above mentioned results it could be conclude that table eggs from Balady system were highly contaminated with various pathogenic microorganisms which render them unsafe for consumption than the battery system. Controlling most of eggs pathogen by using propionic acid 30% concentration was recommended, which is easily applicable, safe and improve both keeping quality and hatchability of table and fertile eggs Moreover urgent need for a strategy and protocol for applying strict hygienic measures at level of farm production until table use to control pathogen from pass from one generation to the next through fertile eggs.

Author's Contributions

All authors equally contributed in this work.

Ethics

The authors declare no conflict of interests with respect to the research, authorship and/or publication of this paper and has no direct financial relation with the commercial identities mentioned in this study.

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